ESRF	**Experiment title:** **Structural basis for the recognition between lentiviral integrases and LEDGF.**	**Experiment number**: MX-755
Beamline:	**Date of experiment**: from: 20/03/2008 at 16:00 to: 21/03/2008 at 8:00	**Date of report**: 30/04/2008
Shifts:	**Local contact(s)**: Mr. Gianluca CIOCI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Stephen Hare*, Imperial College London, UK

Peter P. Cherepanov*, Imperial College London, UK

Report

Introduction:

LEDGF is a host cell factor involved in HIV (and generally lentiviral) DNA integration (1-4). Lentiviral integrases (INs) and LEDGF interact with high affinity. The host factor engages INs via a small alpha-helical integrase binding domain (IBD) located within its C-terminal region (5-7). On the IN side, the catalytic core domain (CCD) and the N-terminal domain (NTD) have been implicated into the interaction with LEDGF (8). A crystal structure of the HIV-1 IN CCD complexed with LEDGF IBD complex revealed a part of the HIV-1 IN-LEDGF interface (5).

The goals of this experiment:

Our main objective was (*i*) to elucidate the complete interface between HIV IN and LEDGF that involves the NTD and the CCD of the viral protein. We also wanted (*ii*) to obtain more complete structural information about the functional IN complex, and (*iii*) to understand the adaptive changes in lentiviral IN proteins (*e.g.* bovine immunodeficiency virus [BIV] IN) that allow these divergent proteins to recognize a conserved host cell protein.

Experimental method:

We had obtained crystals of two-domain fragments (NTD+CCD) of HIV and BIV INs complexed with LEDGF IBD. The three crystal forms used in the experiment were:

Form	Composition	Crystals size (um)	preliminary diffr. limit
I	HIV IN (1-209)+LEDGF(347-429)	70x70x40	3.6 Å
II	HIV IN (1-209)+LEDGF(347-429)	40x30x30	4 Å
III	BIV IN (1-211)+LEDGF(347-429)	30x10x10	~10 Å

The experiment was very successful. In total, over 30 crystals were tested, and good diffraction data with a very significant gain in resolution were obtained for both HIV-derived crystal forms, although BIV-derived crystals diffracted poorly (8 - 9 Å). The data collection, refined unit cell parameters and scaling statistics for the crystal forms II and I are shown in the table below.

Crystal Form	II	I
Space Group	$P2_12_12_1$	P321
Unit cell parameters	201.4, 202.5, 280.5, 90, 90, 90	210.4 210.4 162.82 90 90 120
Resolution (Å)	50-3.2 (3.37-3.20)	50-3.06 (3.22-3.06)
No. Reflections	188554 (27244)	77620 (11295)
R_{merge}	0.161 (0.562)	0.083 (0.521)
$\langle I/\sigma(I)\rangle$	13.0 (3.9)	16.0 (3.0)
Completeness (%)	100 (100)	99.1 (99.6)
Multiplicity	7.9 (8.0)	6.2 (6.0)

The structure of the HIV-derived complex was solved via molecular replacement using both data sets; the current R_{work}/R_{free} are 19.55/22.2% for form II and 27.2/29.6% for form I. Although crystals of form I diffracted to a higher resolution (3.06 *v.s.* 3.2 Å), the 12-fold NCS present in form II (36 protein chains per ASU) drastically increased the observation:parameter ratio, resulting in a better electron density map and lower R factors (the free reflection set was selected using SHELL option in SFTOOLS). In addition to elucidating the entire IN-IBD interface, these structures revealed a novel tetrameric IN assembly, which might reflect the active state of the enzyme. These structural data are currently being validated using functional assays. We are also working to improve the crystal form III as well as generating alternative crystals to address the adaptive changes in lentiviral integrase proteins that allow these divergent proteins to recognize a conserved host cell factor. This work is supported by the Medical Research Council of the UK.

References:

1. Engelman, A. & Cherepanov, P. (2008) *PLoS Pathog.* 4, e1000046.
2. Shun, M. C., Raghavendra, N. K., Vandegraaff, N., Daigle, J. E., Hughes, S., Kellam, P., Cherepanov, P. & Engelman, A. (2007) *Genes Dev.* 21, 1767-78.
3. Llano, M., Saenz, D. T., Meehan, A., Wongthida, P., Peretz, M., Walker, W. H., Teo, W. & Poeschla, E. M. (2006) *Science* 314, 461-4.
4. Marshall, H. M., Ronen, K., Berry, C., Llano, M., Sutherland, H., Saenz, D., Bickmore, W., Poeschla, E., *et al.* (2007) *PLoS ONE* 2, e1340.
5. Cherepanov, P., Sun, Z. Y., Rahman, S., Maertens, G., Wagner, G. & Engelman, A. (2005) *Nat. Struct. Mol. Biol.* 12, 526-32.
6. Cherepanov, P., Devroe, E., Silver, P. A. & Engelman, A. (2004) *J. Biol. Chem.* 279, 48883-92.
7. Cherepanov, P. (2007) *Nucleic Acids Res.* 35, 113-24.
8. Maertens, G., Cherepanov, P., Pluymers, W., Busschots, K., De Clercq, E., Debyser, Z. & Engelborghs, Y. (2003) *J. Biol. Chem.* 278, 33528-39.